Tumor Microenvironment-Modulating Nanoparticles Boost Chemotherapy Efficacy and Anti-tumor Immunity

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Purpose
Chemotherapy remains the first line treatment for many solid tumors and metastatic cancers but drug resistance and immunosuppression often limit its efficacy leading to treatment failure. Despite various approaches have been attempted to circumvent tumor microenvironment (TME)-associated factors, only few targeted therapeutics have generated positive results in early clinical trials, indicating the complexity of the tumor physiological network and its multifactorial resistance mechanisms. In this work, we develop a new multimodal treatment strategy to modulate multiple drug resistant and immunosuppressive factors in the TME collectively to enhance tumor response to chemotherapy and boost immunity. Previously our laboratory has designed polymer-lipid encapsulated manganese dioxide nanoparticles (PLMD NPs) and demonstrated their effects on reducing tumor hypoxia and neutralizing intratumoral pH by generating oxygen and consuming protons. Herein we investigated whether treatment with PLMD NPs could improve tumor penetration and efficacy of clinically used anticancer drug doxorubicin (DOX) and whether the combination therapy of PLMD+DOX boost antitumor immunity.

Methods
PLMD NPs were prepared by dispersing MnO2 precursor particles in melted lipid and polymer. EMT6 breast tumor cells were cultured under normoxic or hypoxic (1% O2) conditions for 24 h at 37°C and then treated with saline, DOX, or PLMD+DOX in the presence of H2O2 for up to 4 h. Combination therapy effects on TME were further investigated by measuring molecular markers in EMT6 tumor-bearing mice. The efficacy of the combination treatment was evaluated in EMT6 tumor bearing mice by treating the animals IV with free DOX alone, or 4 h after PLMD NP treatment. A re-challenge study was performed by re-inoculating EMT6 cells in cured mice 120 days after the initial treatments. To examine whether the anti-tumor immune response, splenocytes were isolated from all the EMT6 tumor-bearing mice at three weeks post re-challenge study and injected intraperitoneally into naïve Balb/c mice.

Results
PLMD+DOX treatment yielded 2.9 higher DOX uptake and 34% increase in cytotoxicity compared to DOX alone after 4 h treatment under hypoxic conditions. PLMD+DOX treatment also resulted in a 44% decrease in cell proliferation and a 22% (p < 0.05) and 51% (p < 0.05) increase in cell apoptosis and DNA double strand break, respectively. The efficacy study resulted that the median survival time improved by ~ 5.6-fold for mice treated with PLMD+DOX compared to DOX alone. Strikingly PLMD+DOX treatment resulted in 60% cure rate (9 out of 15 mice), while DOX alone showed a 15% cure rate (2 out of 13 mice). This combination therapy also generated anti-tumor immunity against tumor re-inoculation in 88% of surviving mice and provided anti-tumor immunity in naïve mice receiving splenocyte transfer from the surviving mice with 57% of mice without tumor growth after inoculation.

Conclusion
The present work demonstrated that clinically suitable PLMD NPs reproducibly enhanced the efficacy of chemotherapy of anticancer drug DOX with a single dose treatment and showed that the combination therapy is a simple yet powerful approach.